

参考資料

試料（血漿 or 血清）

- 1 試料は血漿か血清か Pittella-Silva, F. et al. Plasma or Serum: Which Is Preferable for Mutation Detection in Liquid Biopsy? Clin. Chem. 66, 946–957 (2020).

分析前プロセス（採血～核酸抽出）

- 2 分析前プロセスのハーモナイゼーション Greytak, S. R. et al. Harmonizing Cell-Free DNA Collection and Processing Practices through Evidence-Based Guidance. Clin. Cancer Res. 26, 3104–3109 (2020).
- 3 肺がん患者血漿、EGFRバリエーションをddPCRで検出する多施設前向き研究、遠心条件・採血後の時間・温度・溶血などに言及 Sorber, L. et al. A Multicenter Study to Assess EGFR Mutational Status in Plasma: Focus on an Optimized Workflow for Liquid Biopsy in a Clinical Setting. Cancers vol. 10 (2018).
- 4 cfDNA, cfRNA採取のための遠心条件の検討 Sorber, L. et al. Circulating Cell-Free DNA and RNA Analysis as Liquid Biopsy: Optimal Centrifugation Protocol. Cancers vol. 11 (2019).
- 5 人工的なcfDNAを用いたテクニカルバリデーション Yang, X. et al. Technical Validation of a Next-Generation Sequencing Assay for Detecting Clinically Relevant Levels of Breast Cancer-Related Single-Nucleotide Variants and Copy Number Variants Using Simulated Cell-Free DNA. J. Mol. Diagnostics 19, 525–536 (2017).
- 6 cfDNA抽出キットの評価 Diefenbach, R. J., Lee, J. H., Kefford, R. F. & Rizos, H. Evaluation of commercial kits for purification of circulating free DNA. Cancer Genet. 228–229, 21–27 (2018).
- 7 cfDNA抽出キットの評価 Solassol, J. et al. Comparison of five cell-free DNA isolation methods to detect the EGFR T790M mutation in plasma samples of patients with lung cancer. Clin. Chem. Lab. Med. 56, e243–e246 (2018).

分析プロセス(分析性能)

- 8 CTC, cfDNA検査における分析前変動要因 Shishido, S. N. et al. Preanalytical Variables for the Genomic Assessment of the Cellular and Acellular Fractions of the Liquid Biopsy in a Cohort of Breast Cancer Patients. J. Mol. Diagnostics 22, 319–337 (2020).
- 9 ctDNA分析法のanalytic validityの評価 202109 Deveson, I. W. et al. Evaluating the analytical validity of circulating tumor DNA sequencing assays for precision oncology. Nat. Biotechnol. 39, 1115–1128 (2021).
- 10 CAPP-seqの論文、NGSをctDNAの解析に用いる。VAFの検出感度を0.02%に向上 Newman, A. M. et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat. Med. 20, 548–554 (2014).
- 11 cfDNAを使って融合遺伝子を検出できるかを検証。Intron部位を含めたWESで解析。ctDNAを用いて、融合遺伝子を検出できることを確認 Lee, J. K. et al. The pan-tumor landscape of targetable kinase fusions in circulating tumor DNA. Clin. Cancer Res. 2136.2021 (2021) doi:10.1158/1078-0432.CCR-21-2136.
- 12 血中に存在するctDNA、ctDNA、エクソソームなどの様々なバイオマーカーを解析する重要性と、その保管方法について Grölz, D. et al. Liquid Biopsy Preservation Solutions for Standardized Pre-Analytical Workflows-Venous Whole Blood and Plasma. Curr. Pathobiol. Rep. 6, 275–286 (2018).

精度管理法

- 13 精度管理法 Samoila, A. et al. Developing Quality Programs for Cell-Free DNA (cfDNA) Extraction from Peripheral Blood. J. Appl. Lab. Med. 5, 788–797 (2020).]
- 14 標準物質 He, H.-J. et al. Multilaboratory Assessment of a New Reference Material for Quality Assurance of Cell-Free Tumor DNA Measurements. J. Mol. Diagnostics 21, 658–676 (2019).
- 15 EQA Van Casteren, K. et al. External Quality Assessment Schemes for Biomarker Testing in Oncology: Comparison of Performance between Formalin-Fixed, Paraffin-Embedded-Tissue and Cell-Free Tumor DNA in Plasma. J. Mol. Diagnostics 22, 736–747 (2020).
- 16 EQA Chai, S. Y. et al. External Quality Assurance of Current Technology for the Testing of Cancer-Associated Circulating Free DNA Variants. Pathol. Oncol. Res. 26, (2019).
- 17 EQA Deans, Z. C. et al. IQN path ASBL report from the first European cfDNA consensus meeting: expert opinion on the minimal requirements for clinical ctDNA testing. Virchows Arch. 474, 681–689 (2019).
- 18 EQA (分析法いろいろ) Haselmann, V. et al. Results of the first external quality assessment scheme (EQA) for isolation and analysis of circulating tumour DNA (ctDNA). Clin. Chem. Lab. Med. 56, 220–228 (2018).
- 19 EQA (分析法いろいろ) Keppens, C. et al. International pilot external quality assessment scheme for analysis and reporting of circulating tumour DNA. BMC Cancer 18, 804 (2018).
- 20 分析・分析後プロセスのEQA、特に報告書 Peng, R. et al. From Somatic Variants Toward Precision Oncology: An Investigation of Reporting Practice for Next-Generation Sequencing-Based Circulating Tumor DNA Analysis. Oncologist 25, 218–228 (2020).

CHIP関連

21 cfDNAとWBCのマッチト解析でCHによるFPを減らすことができる
Razavi, P. et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat. Med.* 25, 1928–1937 (2019).

22 CHはPF以外に他の医学的意味を持つ
Chan, H. T., Chin, Y. M., Nakamura, Y. & Low, S.-K. Clonal Hematopoiesis in Liquid Biopsy: From Biological Noise to Valuable Clinical Implications. *Cancers* vol. 12 (2020).

23 Clonal Hematopoiesisの発生機序に関する総説
Gibson, C. J. & Steensma, D. P. New Insights from Studies of Clonal Hematopoiesis. *Clin. Cancer Res.* 24, 4633–4642 (2018).

24 50000人の血液をシーケンシング、CHの発生、進化と適合性に関する報告
J., W. C. et al. The evolutionary dynamics and fitness landscape of clonal hematopoiesis. *Science* (80), 367, 1449–1454 (2020).

25 cfDNA解析で見つかった変異とからCH由来変異を同定、17%の患者でCH由来変異を同定。
Chan, H. T. et al. Clinical significance of clonal hematopoiesis in the interpretation of blood liquid biopsy. *Mol. Oncol.* 14, 1719–1730 (2020).

26 ctDNA解析の擬陽性の原因となるCHの影響を分析、JAK2変異のほとんど、TP53変異のいくつか、希少なKRAS変異がCH由来。
Hu, Y. et al. False-Positive Plasma Genotyping Due to Clonal Hematopoiesis. *Clin. Cancer Res.* 24, 4437–4443 (2018).

総説

27 総説 (analytic validity to clinical utility)
Cheng, M. L. et al. Circulating tumor DNA in advanced solid tumors: Clinical relevance and future directions. *CA. Cancer J. Clin.* 71, 176–190 (2021).

28 REVIEW 低頻度のバリエーションの検出
Singh, R. R. Next-Generation Sequencing in High-Sensitive Detection of Mutations in Tumors: Challenges, Advances, and Applications. *J. Mol. Diagnostics* 22, 994–1007 (2020).

29 大腸がん治療のためのCGPの臨床ガイドライン (LBも必要)
Ebi, H. et al. Japanese Society of Medical Oncology Clinical Guidelines: Molecular Testing for Colorectal Cancer Treatment, 4th edition. *Cancer Sci.* 111, 3962–3969 (2020).

30 ASCOとCAPによる77報のcfDNA論文を評価したまとめ、cfDNA検査の分析前、分析プロセス、データ解析、報告、臨床的妥当性、臨床的有用性までの全プロセスを対象とする。
Merker, J. D. et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *J. Clin. Oncol.* 36, 1631–1641 (2018).

31 ctDNA解析の総説
Zhao, E. Y. & Bratman, S. V. Emerging Precision Oncology Applications of Liquid Biopsy using Circulating Tumour DNA and Methylome Profiling. *Clin. Oncol.* 32, 626–631 (2020).

32 NSCLCの治療にctDNA解析を利用するための技術的、分析的な問題点について
Abbosh, C., Birkbak, N. J. & Swanton, C. Early stage NSCLC — challenges to implementing ctDNA-based screening and MRD detection. *Nat. Rev. Clin. Oncol.* 15, 577–586 (2018).

ガイドライン

33 癌3学会の政策提言を英文にまとめたもの
Sunami, K. et al. Appropriate use of cancer comprehensive genome profiling assay using circulating tumor DNA. *Cancer Sci.* 112, 3911–3917 (2021)

34 癌3学会の政策提言
日本臨床腫瘍学会・日本癌治療学会・日本癌学会 3学会合同ゲノム医療推進タスクフォース (令和3年1月20) 政策提言 「血中循環腫瘍DNAを用いたがんゲノムプロファイリング検査の適正使用に関する政策提言」 <https://www.jsmo.or.jp/file/dl/newsj/2765.pdf>

35 2013年に作成した肺がんの検査ガイドラインの更新版、18のガイドラインを追加し、3つを更新。
Lindeman, N. I. et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch. Pathol. Lab. Med.* 142, 321–346 (2018).

36 IASLCの肺がんctDNA解析に関する提言
Rolfo, C. et al. Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC. *J. Thorac. Oncol.* 13, 1248–1268 (2018).

組織検査とctDNA検査の臨床的有用性の比較

37 大腸がんにおける組織とctDNAの大規模コホートをを使った初めての比較論文、約1300症例のctDNAを解析して組織の結果と比較、ctDNAの変異検出力は、組織の場合とほぼ同程度。
Strickler, J. H. et al. Genomic Landscape of Cell-Free DNA in Patients with Colorectal Cancer. *Cancer Discov.* 8, 164–173 (2018).

38 治験に参加する胃がん患者のスクリーニング方法として組織とctDNAを使った場合を比較。組織は約5000人、ctDNAは約2000人。ctDNAを使った場合は、組織に比べて、検査結果のTATが1/3(11日対33日)に治験への参加率は約2倍(9.5%対4.1%)に増加した。
Nakamura, Y. et al. Clinical utility of circulating tumor DNA sequencing in advanced gastrointestinal cancer: SCRUM-Japan GI-SCREEN and GOZILA studies. *Nat. Med.* 26, 1859–1864 (2020).

39 公開された論文644報のデータを使って、組織とctDNAの有用性を比較。
Esagian, S. M. et al. Comparison of liquid-based to tissue-based biopsy analysis by targeted next generation sequencing in advanced non-small cell lung cancer: a comprehensive systematic review. *J. Cancer Res. Clin. Oncol.* 146, 2051–2066 (2020).

40 382遺伝子パネルを使ってctDNAを解析。605症例を解析して87%患者からctDNAの変異を検出。組織とctDNAとで検出された変異の一致率は高かった。
Shu, Y. et al. Circulating Tumor DNA Mutation Profiling by Targeted Next Generation Sequencing Provides Guidance for Personalized Treatments in Multiple Cancer Types. *Sci. Rep.* 7, 583 (2017).

41 PCR (cobas)を用いて、ctDNAを解析
Mok, T. et al. Detection and Dynamic Changes of EGFR Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy. *Clin. Cancer Res.* 21, 3196–3203 (2015).

ctDNA検査, cfDNA検査の臨床的有用性、他

42 肝細胞癌のCTC, cfDNA解析の有用性をまとめた総説 Ye, Q., Ling, S., Zheng, S. & Xu, X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. *Mol. Cancer* 18, 114 (2019).

43 CTCやcfDNAを使ったMRDモニターに関する総説 Pantel, K. & Alix-Panabières, C. Liquid biopsy and minimal residual disease — latest advances and implications for cure. *Nat. Rev. Clin. Oncol.* 16, 409–424 (2019).

44 胃がんのがん組織の多様性と薬剤耐性, 再発部位の変異の関係を組織とcfDNAのゲノム解析から比較. cfDNA解析の方が臨床的に有効な結果が得られた. Parikh, A. R. et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat. Med.* 25, 1415–1421 (2019).

45 ctDNAをがんの早期発見に使用できるかについて数学モデルを用いて考察. Stefano, A. et al. A mathematical model of ctDNA shedding predicts tumor detection size. *Sci. Adv.* 6, eabc4308 (2021).

46 dPCRを使ったctDNAの論文. 腫瘍によりctDNAを検出できる割合が異なる. Bettgowda, C. et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. *Sci. Transl. Med.* 6, 224ra24–224ra24 (2014).

47 乳がん患者を対象としたctDNA解析. 患者別にパネルを準備して解析. Yoshinami, T. et al. Detection of ctDNA with Personalized Molecular Barcode NGS and Its Clinical Significance in Patients with Early Breast Cancer. *Transl. Oncol.* 13, 100787 (2020).

48 免疫阻害剤の治療効果の測定にctDNAを応用. Zhang, Q. et al. Prognostic and Predictive Impact of Circulating Tumor DNA in Patients with Advanced Cancers Treated with Immune Checkpoint Blockade. *Cancer Discov.* 10, 1842–1853 (2020).

49 MRD解析には, 多くの場合, 組織のGCP情報が必要とされるが, ctDNA解析のみでMRDに挑戦. Parikh, A. R. et al. Minimal Residual Disease Detection using a Plasma-only Circulating Tumor DNA Assay in Patients with Colorectal Cancer. *Clin. Cancer Res.* (2021) doi:10.1158/1078-0432.CCR-21-0410.

50 Gaurdant360を使った肺がん患者のctDNA解析の結果 Zugazagoitia, J. et al. Clinical utility of plasma-based digital next-generation sequencing in patients with advance-stage lung adenocarcinomas with insufficient tumor samples for tissue genotyping. *Ann. Oncol.* 30, 290–296 (2019).

51 CTCとctDNAを使用した大腸がん患者の予後観察 Sun, Q., Liu, Y., Liu, B. & Liu, Y. Use of Liquid Biopsy in Monitoring Colorectal Cancer Progression Shows Strong Clinical Correlation. *Am. J. Med. Sci.* 355, 220–227 (2018).

52 ctDNA解析でEGFR-TKIの効果を予測可能であった. Iwama, E. et al. Longitudinal monitoring of somatic genetic alterations in circulating cell-free DNA during treatment with epidermal growth factor receptor-tyrosine kinase inhibitors. *Cancer* 126, 219–227 (2020).

53 ctDNA解析でALK変異治療薬の耐性を検出. 治療薬の変更に応用. Dagogo-Jack, I. et al. Treatment with Next-Generation ALK Inhibitors Fuels Plasma ALK Mutation Diversity. *Clin. Cancer Res.* 25, 6662–6670 (2019).

54 ctDNAでALK治療薬の効果と耐性出現を観察 Horn, L. et al. Monitoring Therapeutic Response and Resistance: Analysis of Circulating Tumor DNA in Patients With ALK+ Lung Cancer. *J. Thorac. Oncol.* 14, 1901–1911 (2019).

55 ctDNA量と腫瘍量との関係 Lam, V. K. et al. Genotype-Specific Differences in Circulating Tumor DNA Levels in Advanced NSCLC. *J. Thorac. Oncol.* 16, 601–609 (2021).

56 ctDNA解析で, osimertinibの治療効果予測 Sakai, K. et al. Predicting osimertinib-treatment outcomes through EGFR mutant-fraction monitoring in the circulating tumor DNA of EGFR T790M-positive patients with non-small cell lung cancer (WJOG8815L). *Mol. Oncol.* 15, 126–137 (2021).

57 初期乳がん患者のctDNA解析 Zhang, X. et al. Parallel Analyses of Somatic Mutations in Plasma Circulating Tumor DNA (ctDNA) and Matched Tumor Tissues in Early-Stage Breast Cancer. *Clin. Cancer Res.* 25, 6546–6553 (2019).

58 Oncomine Pan-Cancer cell free assayを使用した乳がん患者のctDNA解析結果 Chin, Y. M. et al. Ultradeep targeted sequencing of circulating tumor DNA in plasma of early and advanced breast cancer. *Cancer Sci.* 112, 454–464 (2021).